FIG. 1A

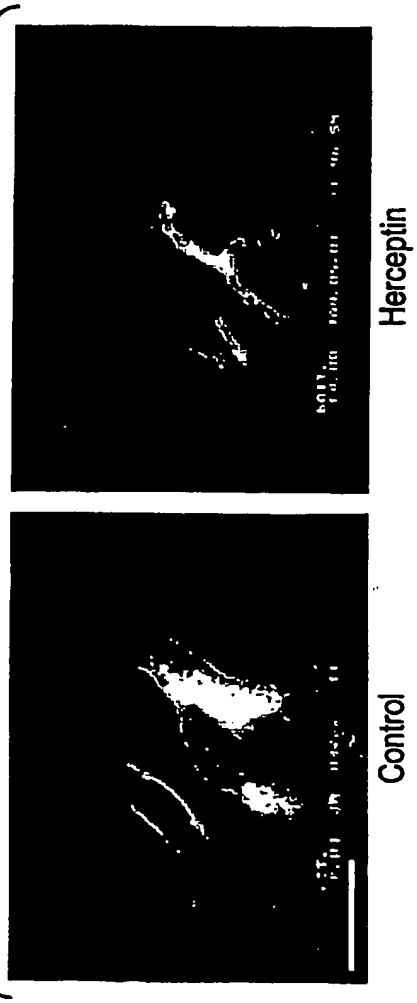

FIG. 1B

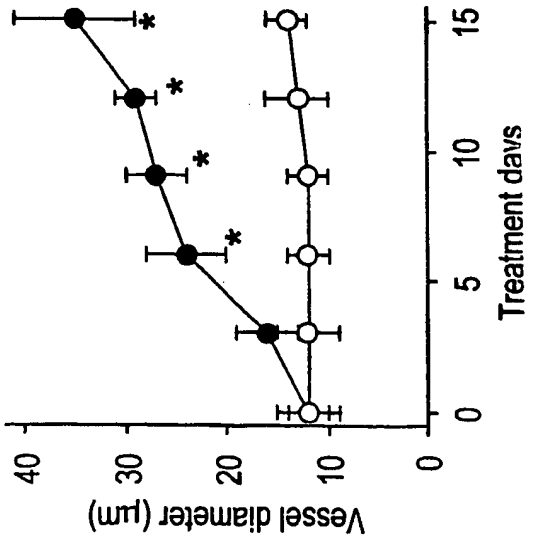

FIG. 1C

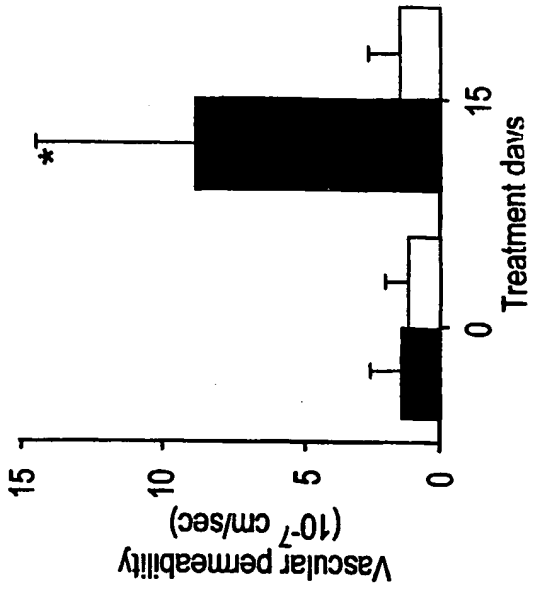

FIG. 1E

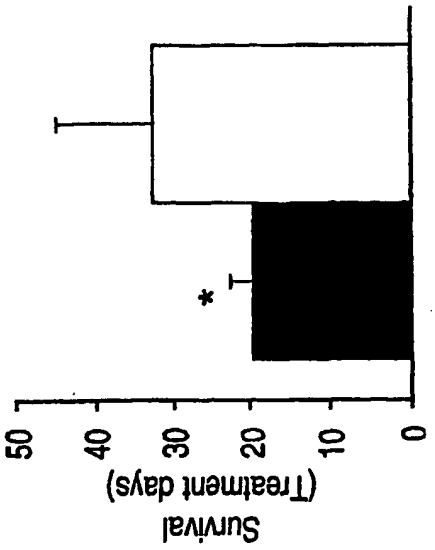

FIG. 1D

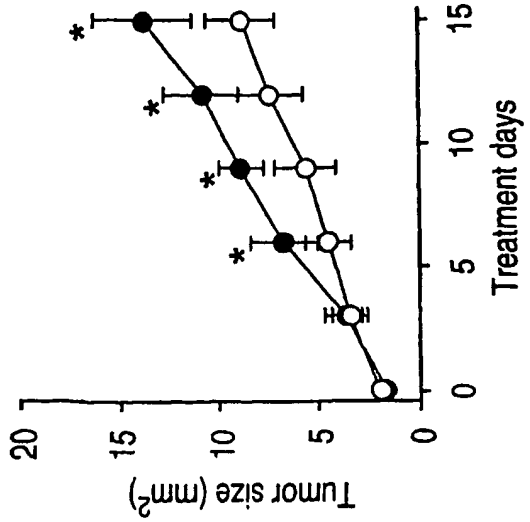

FIG. 1F

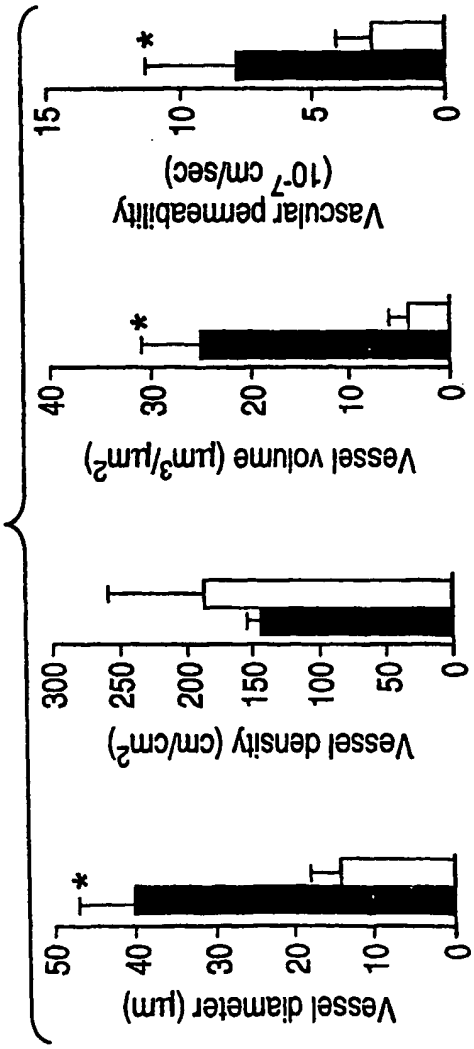

FIG. 1G

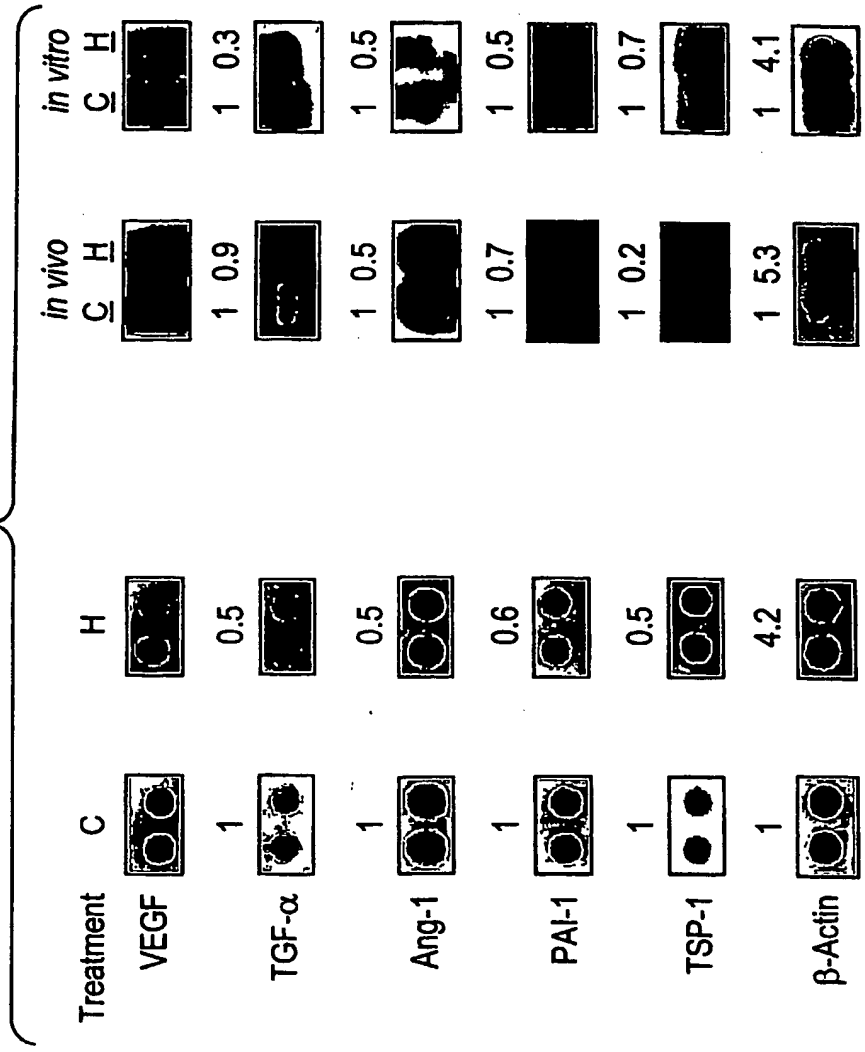
Treatment
C
H
in vivo
C H
in vitro
C H
VEGF
1 0.5
1 0.9
1 0.3
TGF-α
1 0.5
1 0.5
1 0.5
Ang-1
1 0.6
1 0.7
1 0.5
PAI-1
1 0.5
1 0.2
1 0.7
TSP-1
1 4.2
1 5.3
1 4.1
β-Actin

FIG. 1I

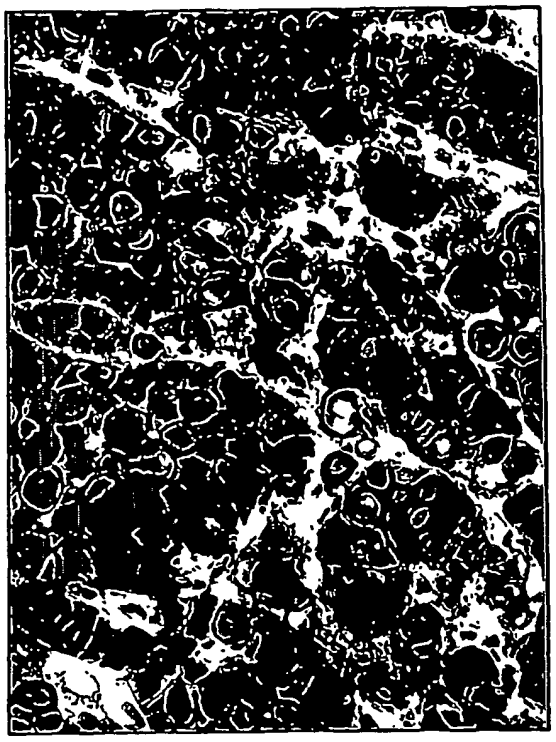

FIG. 1H

FIG. 1J

	Vessel diameter (μm)	Vessel density (cm/cm^2)	Vessel volume ($\mu m^3/\mu m^2$)	Permeability (10^{-7} cm/s)	Survival (days)
Control	40.4 ± 6.7	144 ± 11	25.4 ± 6.3	8.0 ± 3.4	20 ± 3
Herceptin	14.2 ± 4.1*	181 ± 70	3.8 ± 2.3*	2.7 ± 1.4*	33 ± 12*
Gene expression	VEGF	TGFα	Ang-1	PAI-1	TSP-1
Gene array *in vivo*	0.5	0.5	0.6	0.5	4.2
Northern *in vivo*	0.9	0.5	0.7	0.2	5.3
Northern *in vitro*	0.3	0.5	0.5	0.7	4.1

FIG. 2A

Angiogenesis inhibitors in clinical trials for cancer

Drug	Mechanism
Phase I	
EMD121974	Small molecule integrin antagonist
Combretastatin A-4 prodrug	Apoptosis in proliferating endothelium
PTK787/ZK2284	Blocks VEGF-receptor signaling
Endostatin	Induces endotheliail cell apoptosis *in vivo*
BMS-275291	Synthetic MMP inhibitor
SU6668	Blocks VEGF-, FGF-, and PDGF- receptor signaling
Phase II	
CAI	Inhibitor of calcium influx
Squalamine	Inhibits Na^+/H^+ exchanger
COL-3	Synthetic MMP inhibitor; tetracycline derivative
CGS-27023A	Synthetic MMP inhibitor
TNP-470	Fumagilin analogue; inhibits endothelial proliferation
Vitaxin	Antibody to integrin on endothelial surface
IL-12	Induces interferon-γ and IP-10
Anti-VEGF Ab	Monoclonal antibody to VEGF
Phase III	
SU5416	Blocks VEGF receptor signaling
Thalidomide	Unknown
Marimastat	Synthetic MMP inhibitor
AG3340	Synthetic MMP inhibitor
Neovastat	Natural MMP inhibitor
Interferon-α	Inhibition of bFGF and VEGF production
IM862	Unknown mechanism

From NCI Database www.cancertrials.ncl.nih.gov (updated 12 April 2000)

FIG. 2B

Farnesyltransferase Inhibitors in Clinical Development		
Farnesyltransferase Inhibitor	Route of Administration	Phase of Development
R115777	Oral	Phase III
BMS-214662	Oral or IV	Phase I
SCH6636	Oral	Phase II
L-778,123	IV	Phase I

FIG. 2C

Completed and Active Clinical Studies With INGN 201

Phase	Disease	Route of Administration Of Gene Therapy	Study Status
I	Head and neck	IT	Completed (no MTD)[20]
	Non-small cell lung	IT	Completed (no MTD)[23,24]
	Prostate	I Pros	Completed (no MTD)[27]
	Solid tumors	IV	Ongoing
	Ovarian	IP	Ongoing (2 studies)[28]
	Breast	IT	Ongoing
	Bladder	I Vesc	Ongoing
	Brain	IT	Ongoing[29]
	Lung	BAL	Ongoing
II	Head and neck	IT	Completed (2 studies)[21]
	Non-small cell lung	IT	Ongoing[25]
III	Head and neck (single agent)	IT	Ongoing
	Head and neck (+ cisplatin/5-FU)	IT	Ongoing

Abbreviations: IT, intratumoral; I Pros, Intraprostatic; IV, Intravenous; IP, Intraperitoneal; I Vesc, intravesical Instillation; BAL, bronchopulmonary lavage; MTD, maximum tolerated dose; 5-FU, 5-fluorouracil.

FIG. 2D

Potential Indications, Adverse Events, and Trial Status of Drugs Targeting the ErbB Receptor

Drug	Potential Indications	Major Adverse Events
Monoclonal Antibodies		
Trastuzumab	ErbB2-overexpressing metastatic breast cancer (FDA approved); Other erbB2-driven tumors	Fever, chills, pain, dyspnea; cardiotoxicity, especially when combined with cytotoxic drugs
C225	EGFR-driven tumors, especially head and neck	Fever, chills, asthenia, nausea, acneiform, rash
MDX-H210	erbB2-driven tumors	Acute reactions to IV infusion
MDX-447	EGFR-driven tumors	Hypotension
Tyrosine Kinase Inhibitors		
ZD1839	EGFR-driven tumors, especially NSCLC	Rash, diarrhea, nausea, vomiting
OSI-774	EGFR-driven tumors, especially NSCLC	Fatigue, headache, nausea, diarrhea, rash
CI-1033	Tumors driven by any one or multiple erbB receptors	NA
PK1-166	EGFR-driven tumors	NA

Abbreviations: NSCLC, non-small cell lung cancer; IV, intravenous; FDA, Food and Drug Administration

FIG. 2E

Small-Molecule Inhibitors of the EGFR Kinase Currently in Preclinical and Clinical Development

Small Molecule	EGFR IC_{50} (μmol/L)*	HER2 IC_{50} (μmol/L)	Reference(s)
AG-1478	<0.003	1.4+	5.25
AG-1517¶	0.0009	Not reported	25
PD153035¶	0.029 ± 0.005	2.3+	28
ZD1839	0.033	>3.7++	29
OSI-774	0.02	Not reported	30
PD168393#	0.0007 ± 0.00009	5.7 ± 0.8§	23
PD158780	0.000008	0.05‖	24

*Most values reflect the IC_{50} using purified EGFR in vitro as a substrate
+Personal communication, Laura Shawver, Sugen, Inc (South San Fancisco, CA), 1998.
++Effect purified HER2 kinase in vitro
§Effect on heregulin-mediated phosphorylation (of HER2).
‖Effect on heregulin-stimulated phosphorylation (of HER2) in SKBR-3 and MDA-453 cells.
¶These two quinazolines have the same structure (refs 4 and 28)
#Only reported irreversible inhibitor.

FIG. 2F-1

Drugs that block matrix breakdown:				
Drug	Sponsor	Trial	Mechanism	For More Info:
Marimastat	British Biotech	Phase III small cell lung cancers	Synthetic inhibitor of matrix metalloproteinases (MMPs)	800-4-CANCER Online information
COL-3	Collagenex; Newtown, PA	Phase I/II brain, Kaposi's Sarcoma	Synthetic MMP inhibitor Tetracycline® derivative	800-4-CANCER Online information
Neovastat	Aeterna; Québec	Phase II Multiple Myeloma, Phase III renal cell (kidney) cancer, Phase III non-small cell lung cancer	Naturally occurring MMP inhibitor	888-349-3232 Online information
BMS-275291	Bristol-Myers Squibb; Wallingford, CT	Phase I/II Kaposi's sarcoma, Phase II/III Advanced or Metastatic Non-Small Cell Lung	Synthetic MMP inhibitor	203-677-6779 Online information

Drugs that inhibit endothelial cells directly:				
Drug	Sponsor	Trial	Mechanism	For More Info:
Thalidomide	Commercially available, approved for leprosy, Celgene	Phase I Malignant Glioma, Phase I/II for advanced Melanoma, Phase II ovarian, metastatic prostate, Phase II with chemotherapy against solid tumors; adjuvant study in recurrent or metastatic colorectal cancer; Myelofibrosis with myeloid metaplasia, follicular lymphoma, myelodysplastic syndrome, refractory ovarian, Phase II gynecologic sarcomas, liver cancer, metastatic melanoma, CLL, Multiple Myeloma, Phase III non-small cell lung, nonmetastatic prostate, refractory multiple myeloma, renal cancer.	Unknown	732-805-3905 or 800-890-4619 ext. 3905 or 800-4-CANCER or 1-888-NCI-1937Online Information
Squalamine	Genera Pharmaceuticals; Plymouth Meeting, PA	Phase II non small cell lung cancer; Phase II Ovarian; Brain; Phase I Advanced Cancers	Extract from dogfish shark liver; inhibits sodium-hydrogen exchanger, NHE3	610-941-4020 or 800-4-CANCER Online information
2-ME	EntreMed, Rockville, MD	Phase I solid tumor studies	Inhibition of endothelial cells	800-4-CANCER Online information

FIG. 2F-2

Drugs that block activators of angiogenesis:				
Drug	Sponsor	Trial	Mechanism	For More Info:
SU6668	Sugen, South San Francisco, CA	Phase I against advanced tumors	Blocks VEGF, FGF, and PDGF receptor signaling	800-SUGEN-06 or 650-553-8678 Online Information (Link to Sugen Web site)
Interferon-alpha	Commercially available	Phase II/III (search* NCI trials database for listings)	Inhibition of bFGF and VEGF production	800-4-CANCER or 888-NCI-1937
Anti-VEGF Antibody	National Cancer Institute, Bethesda, MD; Genentech, San Francsco, CA	Advanced head and neck. Phase II metastatic renal cell cancer, Phase II with chemotherapy in untreated advanced colorectal, metastatic breast; Phase II non-hodgkin's lymphoma, hematologic malignancies, metastatic prostate, previously untreated advanced colorectal, inflammatory breast cancer, Advanced or recurrent cervical, non-small cell lung; Phase II/III Advanced non-small cell lung; Phase III with chemotherapy in untreated metastatic colorectal, Phase III metastatic breast	Monoclonal antibody to vascular endothelial growth factor (VEGF)	888-624-1937or 800-4-CANCER Online Information

* When searching for interferon-alpha trials in the NCI database, select "cytokine therapy" rather than "antiangiogenesis therapy" in the modality field.

Drugs that inhibit endothelial-specific integrin/survival signaling:				
Drug	Sponsor	Trial	Mechanism	For More Info:
Medi-522 (Vitaxin II)	MedImmune, Inc., Gaithersburg, Maryland	Phase I/II trial in CPT-11 (irinotecan) refractory advanced colorectal cancer	Antibody that blocks the integrin present on endothelial cell surface	800-4-CANCER Online Information
EMD121974	Merck KCgaA; Darmstadt, Germany	Phase I in patients with HIV related Kaposi's Sarcoma, Phase I/II progressive or recurrent Anaplastic Glioma	Small molecule blocker of integrin present on endothelial cell surface	800-4-CANCER Online Information

FIG. 2F-3

Drugs with non-specific mechanism of action:				
Drug	Sponsor	Trial	Mechanism	For More Info:
[illegible]AI	National Cancer Institute, Bethesda, MD	Phase I studies in combination against solid tumors, Phase II ovarian cancer, metastatic renal cell cancer	Inhibitor of calcium influx	800-4-CANCER or 888-624-1937 Online information
[illegible]alecoxib	Pharmacia	Phase I Prostate; Phase I/II Cervical; Phase II Basal Cell, Metastatic Breast	Enxyme cyclo-oxygenase 2 (COX-2)	800-4-CANCER Online information
[illegible]terleukin-12	Genetics Institute; Cambridge, MA	Phase I/II Kaposi's sarcoma	Up-regulation of interferon gamma and IP-10	800-4-CANCER Online information
[illegible]962	Cytran; Kirkland, WA	Phase II for untreated metastatic cancers of the colon and rectum; Ovarian	Unknown mechanism	425-889-5808 or 800 4-CANCER Online information

FIG. 2G-1

Tyrosine kinase inhibitor

1. ZD1839
2. Cetuximab
3. STI571
4. SU5416
5. SU6668
6. Phenoxodiol
7. Imatinib Mesylate
8. Erlotin ib
9. OSI-774
10. USN-01

Enzyme inhibitor

1. PS-341
2. ISIS3521
3. AG2037
4. Imatinib Mesylate
5. STI571
6. ZD1839
7. R115777
8. SCH66336
9. Phenoxodil
10. SU5416
11. Celecoxib
12. Erlotinib
13. Trastuzumab
14. OSI-774
15. Paclitaxel, Lometrexol

Other signaling inhibitors

1. Perifosine: alkylphospholipid modulator of signal transduction
2. Flavopiridole: cyclin-dependent kinase inhibitor
3. Genasense (G3139): Bcl-2 antisense
4. Ras peptide vaccine
5. P53 peptide vaccine
6. VHL peptide

FIG. 2G-2

Antibody therapy

1. BEC2
2. CD20 (Rituximab)
3. ch14.18
4. CD52 (Campath-1H)
5. ABX-CBL
6. Edrecolomab
7. Trastuzumab
8. m170
9. Lym-1
10. BrE-3
11. M195
12. Bevacizumab: rhuMAb VEGF
13. Tositumomab
14. 3F8
15. HMFG1
16. CC49-deltaCH2
17. IDEC-Y2B8 (Ibritumomab tiuxetan)
18. IDEC-In2B8
19. Hu3S193
20. HeFi-1
21. 81C6
22. Hu1D10 (Apolizumab)
23. ABX-EGF
24. HuM291
25. 4G7xH22
26. MN-14
27. huJ591
28. 105AD7
29. SGN-15 (cBR96-doxorubicin immunoconjugate)
30. Gemtuzumab ozogamicin
31. MDX-CTLA4
32. Zenapax (daclizumab, anti-Tac): anti-IL2 receptor alpha
33. Cetuximab
34. OKT3
35. Epratuzumab
36. TNT-1/B
37. MDX447
38. IL-13 PE38QQR immunotoxin
39. LMB-9 immunotoxin
40. MIK-Beta-1
41. I131 anti-B1 antibody
42. Cereport: anti-brain capillary endothelial cell B2 receptor

FIG. 2G-3

Hormonal

1. Letrozole: antiestrogen
2. Anastrozole: endocrine therapy
3. Raloxifene: anti-estrogen
4. Medroxyprogesterone: progesterone therapy
5. Bicalutamide: anti-androgen

Cytokine related

1. Anti-thymocyte globulin and TNF receptor IgG chimera: anti-cytokine, biological response modifier
2. Filgrastim (G-CSF): cytokine therapy
3. Enbrel (Tumor necrosis factor fusion protein): anti-cytokine
4. IL13-PE38QQR: IL13 + PE38QQR (bacteria toxin), cell kill against IL13 positive tumor cells
5. Etanercept: soluble TNF alpha receptor, anti-inflammatory
6. Interferon alpha: inhibit renal cell growth, immunotherapy
7. F1t3L: cytokine therapy
8. Sargramostim: cytokine
9. Infliximab: anti-TNF alpha
10. Azacitidine: cytrokine
11. Amifostine: cytrokine

Growth factor antagonist

1. Temozolomide
2. Deltaparin
3. EMD121974
4. CC-5013: Thalidomide analogue
5. RPI4610
6. Shark cartilage extract

Immune modifier

1. APC8015
2. BMS-275291

FIG. 2G-4

Chemosensitizer

1. PSC 833: drug resistance inhibition
2. Bryostatin 1: chemosensitizer
3. UCN-01: decrease tumor threshold for apoptosis

Chemoprevention

1. Eflornithine: chemoprevention
2. Sulindac: chemoprevention
3. LY353381

Others

1. Goserelin: releasing factor agonist
2. Exemestane: aromatase inhibition
3. Tretinoin: normalize renal cancer cells

FIG. 3

Angiogenesis activators and inhibitors

Activators	Function	Inhibitors	Function
VEGF family Members (+) (#)	Stimulate angio / vasculogenesis, permeability, leukocyte adhesion	VEGFR-1; soluble VEGFR-1 soluble neuropilin-1 (NRP-1)	Sink for VEGF, VEGF-B, PlGF
VEGFR (#), NRP-I, NRP-2	Integrate angiogenic and survival signals	Ang2(#)(*)	Antagonist of Ang1
EG-VEGF	Stimulate growth of endothelial cells derived from endocrine glands	TSP-1,2	Inhibit endothelial migration, growth, adhesion & survival
Ang1 and Tie2 (+)(#)	Stabilize vessels	Angiostatin and related plasminogen kringles	Inhibit endothelial migration and survival
PDGF-BB and Receptors	Recruit smooth muscle cells	Endostatin (Collagen XVIII fragment)	Inhibit endothelial survival and migration
TGF-β1 (*),endoglin, TGF- β receptors	Stimulate extracellular matrix production	Tumstatin	Inhibit endothelial protein synthesis
FGF, HGF, MCP-1	Stimulate angio/arteriogenesis	Vasostatin; calreticulin	Inhibit endothelial growth
Integrins αv β3(*),αv β5,α β1	Receptors for matrix macromolecules and proteinases	Platelet factor-4	Inhibit binding of bFGF and VEGF
VE-cadherin; PECAM (CD31)	Endothelial junctional molecules	Tissue-inhibitors of MMP(TIMPs): MMP-inhibitors; PEX	Suppress pathologic angiogenesis
Ephrins(#)	Regulate arterial / venous specification	Meth-1, Meth-2	Inhibitors containing MMP-, TSP-, and disintegrin-domains
Plasminogen Activators, MMPs	Remodel matrix, release growth factor	IFN-α, - β, -γ, IP-10, IL-4, IL-12, IL-18	Inhibit endothelial migration; downregulate bFGF
PAI-1	Stabilize nascent vessels	Prothrombin kringle-2; anti-thrombin III fragment	Suppress endothelial growth
NOS; COX-2	Stimulate angiogenesis and vasodilation	16 kD-prolactin	Inhibit bFGF/VEGF
AC133	Regulate angioblast differentiation	VEGI	Modulate cell growth
Chemokines (*)	Pleiotropic role in angiogenesis	Fragment of SPARC	Inhibit endothelial binding and activity of VEGF
Id1/Id3	Inhibit differentiation	Osteopontin fragment	Interfere with intergrin signaling
		Maspin	Protease inhibitor
		Canstatin, Proliferin-related protein, Restin	Mechanisms unknown

For complete function and references, see supplementary information (http://steele.mgh.harvard.edu); (*): opposite effect in some contexts; (+): also present in or affecting non-endothelial cells.

FIG. 4

Angiogenesis in neoplasms and other diseases			
gan	Processes characterized by abnormal angiogenesis or vascular malfunction*	Organ	Processes characterized by abnormal angiogenesis or vascular malfunction*
ad vessels	†Atherosclerosis, haemangioma, haemangioendothelioma, §Vascular malformations	Bone, joints	†Rheumatoid arthritis, synovitis, bone and cartilage destruction osteomyelitis, pannus growth, osteophyte formation, cancer ‡Aseptic necrosis, impaired healing of fractures
	†Warts, pyogenic granulomas, hair growth, Kaposi's sarcoma, scar keloids, allergic oedema, neoplasms §Psoriasis (skin vessels enlarge and become tortuous) ‡Decubitus or stasis ulcers, gastrointestinal ulcers	Liver, kindney, lung, ear and other epithelia	†Inflammatory and infectious processes (hepatitis, pneumonia glomerulonephritis), asthma, nasal polyps, transplantation, liver regeneration, cancer §Pulmonary hypertension, diabetes ‡Pulmonary and systemic hypertension (vascular pruning)
rus, ovary, enta	†Dysfunctional uterine bleeding (contraception), folluicular cysts, ovarian hyperstimulation, endometriosis, neoplasms §Pre-eclampsia ‡Placental insufficiency	Brain, nerves, eye	†Retinopathy of prematurity, diabetic retinopathy, choroidal and other instraocular disorders, leukomalacia, cancer ‡Stroke vascular dementia, Alzheimer's disease, CADASIL
itoneum, pleura	‖Respiratory distress, ascites, peritoneal sclerosis (dialysis patients), adhesion formation (abdominal surgery),metastatic spreading	Endocrine organs	†Thyroiditis, thryroid enlargement, pancreas transplantation ‡Thyroid pseudocyst
n, skeletal cle	†Work overload ‡Ischaemic heart and limb disease	Lymph vessels	†Tumor metastasis, lymphoproliferative disorders ‡Lymphoedema
ose tissue	†Obesity	Haematopoiesis	†AIDS (Kaposi), haematologic malignancies

t of selected examples

reased vascularization; ‡Insufficient vascularization; §abnormal remodeling; ‖increased vascularization and/or permeability; see text for abbreviations